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Research paper

Expression of mRNA for multiple serotonin (5-HT) receptor types/subtypes by the peripheral blood mononuclear cells of rhesus macaques

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Abstract

To find out whether rhesus macaque peripheral blood mononuclear cells (PBMCs) express mRNA for 5-HT receptors, blood samples from normal healthy rhesus monkeys were used to isolate PBMCs by Ficoll-paque density gradient centrifugation. Total RNA was extracted from MT-2 cells, Hut-78 cells, naive or phytohemagglutinin (PHA) stimulated human and monkey PBMCs. One tube RT-PCR was performed using primers specific for human 5-HT1_A, 5-HT1_B, 5-HT1_E, 5-HT2_A, 5-HT2_B, 5-HT2_C, 5-HT3, 5-HT4, 5-HT6, and 5-HT7 receptors. Amplicons of expected sizes were obtained from human cell lines as well as both human and monkey PBMCs. Both PHA stimulated human and monkey PBMCs express mRNAs for 5-HT1_A, 5-HT1_B, 5-HT1_E, 5-HT2_A, 5-HT3, 5-HT4, 5-HT6 receptor types/subtypes. However, mRNAs for 5-HT1_B, 5-HT1_E and 5-HT2_A cannot be confidently detected in some of the PBMC samples without PHA stimulation. 5-HT2_B and 5-HT7 receptor mRNA was not detected in most of the samples and 5-HT2_C receptor mNRA was not detected at all. FACS analysis revealed that CD3+ lymphocyte increased more than 20% among lymphocytes in the PHA stimulated PBMCs. These data indicate that similar to human PBMC, rhesus macaque PBMC may express multiple types of 5-HT receptors and the expression profile could change after PHA stimulation due to either the changes in cell composition or changes in gene transcription level. This provided a basis for further studies on the neuroimmunomodulatory interactions of 5-HT in rhesus macaques.

Keywords: Serotonin; 5-HT receptors; One-step RT-PCR; PBMCs; *Macaca mulatta*

1. Introduction

Serotonin (5-hydroxytryptamine, 5-HT) is a biogenic monoamine with a variety of functions in many physiological and pathophysiological processes such as depression and suicide, anxiety, sleep and circadian rhythm, aggression, feeding and sexual behaviors, schizophrenia, bulimia nervosa and anorexia nervosa, asthma, etc. (Kawka, 1967; Gershon, 1968; Weight and Salmoiraghi, 1968; Koella, 1969; Thompson, 1971; Harvey et al., 1975; Meltzer et al., 1982; Walker and Codd, 1985; Cowen et al., 1990; Morin et al., 1990; Murphy et al., 1991; Wolfe et al., 1997; Goadsby, 1998; Lucki, 1998; Egermayer et al., 1999; Jouvet, 1999; Morin, 1999; Cazzola and Matera, 2000; Buznikov et al., 2001; etc.). Abnormal 5-HT level or metabolism were also observed in acquired immunodeficiency syndrome (AIDS) patients (Launay et al., 1989) which may be related to the neuropsychological disorders observed in HIV/AIDS. In recent years it has been demonstrated that 5-HT is one of the key player in the neuro-endocrine-immune networks due to its ubiquitous distribution and various functions in the nervous system, the endocrine system and the immune system (Koella, 1969; Yang, 1997; Mossner and Lesch, 1998; Barkhudaryan and Dunn, 1999; Raap and Van de Kar, 1999; Grimaldi and Fillion, 2000; Kim and Camilleri, 2000; Hanley and Van de Kar, 2003). More than 90% of the body 5-HT is located in the gut endocrine cells, i.e. enterochromaffin (EC) cells (Racke et al., 1996). In a recent study, we have shown that some of the T and B lymphocytes were in contact with or in close proximity to 5-HT secreting

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enteroendocrine cells or their cytoplasmic processes in the gut mucosa of rhesus monkeys (Yang and Lackner, 2004). However, knowledge concerning the role of 5-HT in the regulation of macaque immune responses is very scanty.

It is well established that various biological effects of 5-HT are mediated through different serotonin receptors and their signal transduction pathways (Sternberg et al., 1986; Gothert and Schlicker, 1987; Hoyer, 1990; Roth et al., 1990; Shih and Chen, 1990; Tecott and Julius, 1993; Peroutka, 1994; Gerhardt and van Heerikhuizen, 1997; Bagdy, 1998; Hasler, 1999; De Vry and Schreiber, 2000; Cappelli et al., 2002; Noda et al., 2004). There are about 7 classes of serotonin receptors that have been documented in the 5-HT receptor family and some of them are further divided into several subtypes (Hoyer and Martin, 1996; Farber et al., 2004). Except for 5-HT type 3 receptors, which are ligand-gated cation channels, all the other 5-HT receptor types/subtypes are G-protein coupled receptors. If 5-HT can affect the immune responses of rhesus macaques, mRNAs for at least one type/subtype of these 5-HT receptors are expected to be detected in rhesus macaque PBMCs.

Up to now the counterpart of human 5-HT receptors has been documented in macaques (Hoyer et al., 1990; Gundlah et al., 1999; Alberts et al., 2000; Salli et al., 2004), although most of these studies have been focused on 5-HT receptors and their functions in the nervous system and reproductive system, etc., with very few if any reports on 5-HT receptors expressed by the immune cells of rhesus macaques. Since several 5-HT receptor types/subtypes have been reported on human immunocytes, such as lymphocytes, monocytes and dendritic cells (Aune et al., 1993; Heidmann et al., 1997; Idzko et al., 2004; Durk et al., 2005), we expected to find 5-HT receptor gene products in rhesus macaque peripheral blood mononuclear cells because of the close genetic distances. By one-step reverse transcription polymerase chain reaction (RT-PCR), we examined the presence of mRNAs for 10 of the 5-HT receptor types/subtypes in the rhesus macaque PBMCs in comparison to that in human PBMCs and cell lines. As expected mRNAs for 5-HT receptors, i.e. 5-HT1_A, 5-HT1_B, 5-HT1_E, 5-HT2_A, 5-HT3, 5-HT4, 5-HT6 were consistently detected in the rhesus macaque PBMCs and the relative level of mRNAs for some 5-HT receptor subtypes was probably affected by PHA stimulation.

2. Materials and methods

2.1. Purification of PBMCs

Monkey PBMCs were isolated from whole blood withdrawn from 4 normal rhesus macaques (*Macaca mulatta*) (animal number: 035, 368, 426 and 318). Human PBMCs were isolated from buffy coat obtained from a blood station where blood was collected from healthy human blood donors. Both monkey and human PBMCs were isolated by density gradient centrifugation on Ficoll-Paque Plus lymphocyte separation medium (Amersham Biosciences, Sweden). The layer with PBMCs was removed and washed twice with sterile phosphate buffered saline (PBS) and some of the recovered cells were used directly for total RNA isolation, the rest of them were used for PHA stimulation.

2.2. Cell culture

Human and monkey PBMCs, human T-cell leukemia cell MT-2 and human cutaneous T cell lymphoma cell Hut-78 were cultivated with HyQ RPMI 1640 medium (Hyclone, USA), containing 10% fetal bovine serum (FBS), 1% L-glutamine, 100 IU/ml penicillin and 100 μg/ml streptomycin at 37 °C in a humidified atmosphere with 5% CO_2. To stimulate the human and monkey PBMCs phytohemagglutinin (PHA, final concentration about 2.5 μg/ml) and IL-2 (final concentration, 20 U/ml) were added to each PBMC culture and kept in the CO_2 incubator for 3 days.

2.3. Flow cytometry

Percentage of lymphocytes, monocytes and granulocytes in the white blood cells was analyzed using a hemocytometer, Celltac X (Nihon Kohden, Japan). CD3, CD4 and CD8 surface makers were examined by flow cytometry. Briefly, whole blood samples from rhesus macaques were stained with appropriate fluorochrome labeled anti-CD3, anti-CD4 and anti-CD8 antibodies (Pharmingen, USA) for 30 min. Red blood cells were then lysed with red cell lysing buffer and samples were analyzed on flow cytometer, FACScalibaur (BD Biosciences, USA). PHA stimulated PBMCs were collected after 3 days of incubation and were stained with the same set of antibodies, washed and analyzed using the same flow cytometer. All the collected data were analyzed using BD CellQuest pro software.

2.4. Isolation of RNA and RT-PCR

Total cellular RNA was extracted from PBMCs and MT-2 and Hut-78 cells using the RNeasy Mini Kit (Qiagen, Germany) according to the manufacturer's instructions.

To examine the presence of 5-HT receptor gene transcripts, 2 μl of extracted RNA was used as template and Takara One-Step RNA PCR Kits (AMV) (TaKaRa Biotechnology, Japan) were used to set up the reaction system according to the manufacturer's instructions. 5-HT1_A, 5-HT1_B, 5-HT1_E, 5-HT2_A, 5-HT2_B, 5-HT2_C, 5-HT3, 5-HT4, 5-HT6, 5-HT7 and β_2-microglobulin primer sequences and PCR cycling conditions were the same as previously reported (Idzko et al., 2004; Durk et al., 2005) except for the 30 min reverse transcription before the thirty PCR cycles, i.e. 50 °C for 30 min, 94 °C for 2 min and then 30 cycles at 94 °C (1 min), 62 °C (1 min) and 72 °C (1 min). All of the one-step RT-PCR cycles were accomplished using the GeneAmp PCR System 9700 (Applied Biosystems, USA). For each sample, the RT-PCR was repeated up to 4 times.